**Photosynthetic adjustment in field-grown ponderosa pine trees after six years
exposure to elevated CO₂**

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Summary Photosynthesis of tree seedlings is generally enhanced during short-term exposure to elevated atmospheric CO₂, but longer-term photosynthetic responses are often more variable because they are affected by morphological, biochemical and physiological feedback mechanisms that regulate carbon assimilation to meet sink demand. In order to examine potential biochemical and morphological factors that might regulate the long-term photosynthetic response of field-grown trees to elevated CO₂, we grew ponderosa pine (*Pinus ponderosa*) trees in open-top chambers for six years in native soil at ambient CO₂ (35 Pa) and elevated CO₂ (70 Pa) at a site near Placerville, CA. Trees were well watered and exposed to natural light and ambient temperature. Net photosynthesis was enhanced 53% in ponderosa pine trees grown in elevated CO₂ at the end of the sixth growing season, despite reductions in photosynthetic capacity. The positive net photosynthetic response of ponderosa pine trees to elevated CO₂ reflected greater relative increases in Rubisco sensitivity than decreases due to biochemical adjustments. Reductions in photosynthetic capacity of elevated CO₂ trees were evident as analyses of A/Ci curves indicated significant reductions in maximum photosynthetic rate ($A_{\max}$, 20%), Rubisco carboxylation capacity (V_{cmax} , 36%), and electron transport capacity ($J_{\max}$, 21%). In addition, the reduction in photosynthetic capacity due to growth in elevated CO₂ was accompanied by reductions in various photosynthetic components, including total chlorophyll (24%), Rubisco protein content (38%), and mass-based leaf nitrogen concentration (14%). Net photosynthesis was unaffected by morphological adjustments since there was no change in leaf mass per unit area in elevated CO₂. An apparent positive response of photosynthetic adjustment in elevated CO₂ was the redistribution of N within the photosynthetic system to balance Rubisco carboxylation and electron

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transport capacities. These results clearly demonstrate that trees, without apparent limitations to root growth, may exhibit photosynthetic adjustment responses in the field after long-term exposure to elevated CO₂. Information from long-term studies on photosynthetic responses in trees will improve our ability to predict annual global carbon influx into terrestrial ecosystems and improve models of forest ecosystem response to global climate change.

Keywords: carbohydrates, carboxylation capacity, electron transport capacity, elevated CO₂, forest trees, global change, nitrogen, photosynthetic adjustment, Pinus ponderosa, Rubisco.

Introduction

Forest trees are major components of the terrestrial biome, and their response to rising atmospheric CO₂ may have a significant effect on the global carbon cycle (Vitousek 1991). Trees are a substantial sink for CO₂, accounting for approximately 70% of terrestrial atmospheric carbon fixation (Waring and Schlesinger 1985), and tree response to rapidly increasing atmospheric CO₂ may ultimately affect the rate of change in CO₂ partial pressure in the atmosphere (Harmon et al. 1990). Knowledge of photosynthetic sensitivity and potential long-term photosynthetic acclimation responses to elevated CO₂ may be used to predict annual global carbon influx into terrestrial ecosystems (Luo et al. 1996) and improve models of forest ecosystem response to global climate change.

The direct short-term stimulation of photosynthesis in trees has been frequently documented (cf. Gunderson and Wullschleger 1994, Curtis 1996) and can be largely described in terms of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) carboxylation efficiency (i.e. carboxylation rate per unit of photosynthetic machinery). Rubisco is CO₂ substrate-limited at current atmospheric conditions. However, carboxylation is stimulated by increased CO₂ partial pressure which increases carboxylation and decreases oxygenation. In fact, a doubling of atmospheric CO₂ partial pressure results in a 50% reduction in the ratio of photorespiration to photosynthesis (Sharkey 1988), thereby increasing net photosynthesis.

Long-term photosynthetic responses to elevated CO₂ are more variable because they are affected by biochemical, morphological and physiological feedback mechanisms that regulate carbon assimilation to meet sink (growth) demand (cf. Stitt 1991, Long and

Drake 1991, Sage 1994, Griffin and Seemann 1996). Reduction in photosynthetic capacity, often termed downregulation or photosynthetic adjustment, after long-term exposure to elevated CO_2 has been observed in trees (Lewis et al. 1996, Atkinson et al. 1997, Rey and Jarvis 1998) and is often attributed to an imbalance in the source-sink relationship due to the accumulation of leaf carbohydrates (Webber et al. 1994, Van Oosten and Besford 1994, Jones et al. 1996, Cheng et al. 1998). Substantial production of carbohydrates induced by higher photosynthetic rates at elevated CO_2 partial pressure initiates a feedback mechanism that ultimately reduces photosynthetic capacity. The production of large amounts of carbohydrates are proposed to lead to reductions in gene transcription and production of Rubisco protein (Sheen 1990, Krapp et al. 1993, Furbank and Taylor 1995, Cheng et al. 1998), which reduces Calvin cycle activity and sugar production, completing the feedback mechanism. This long-term regulation of photosynthesis may occur within 6-9 days after initial exposure to elevated CO_2 (Cheng et al. 1998). Shorter term regulation of photosynthesis may be accomplished by reducing Rubisco activity through decarbamylation of the enzyme (Sage et al. 1988).

In this study, we used a conceptual model of photosynthetic response to elevated CO_2 (Luo et al. 1994, Griffin and Seemann 1996) to examine potential mechanisms that might regulate the long-term photosynthetic response of field-grown ponderosa pine (*Pinus ponderosa*) to elevated atmospheric CO_2 . This model states that photosynthesis at a given growth CO_2 concentration is regulated by three processes: Rubisco sensitivity, biochemical downregulation, and morphological upregulation (Luo et al. 1994). As growth CO_2 increases, the sensitivity of Rubisco to CO_2 partial pressure increases, eventually reaching a plateau, leading to higher photosynthetic rates (Stitt 1991). In

response to elevated growth CO_2 partial pressure, biochemical adjustments may occur, including depressed gene expression, reduced Rubisco content and activity, ribulose biphosphate (RuBP) regeneration limitation, and end-product inhibition regulated by non-structural carbohydrates. These biochemical adjustments generally reduce photosynthetic capacity (i.e. downregulation) and are indicated by changes in mass-based leaf nitrogen concentration. Morphological adjustments, most often observed as increased mesophyll tissue growth (Vu et al. 1989), are indicated by changes in leaf mass per unit area and tend to increase photosynthetic capacity (i.e. upregulation). The relative changes in these three processes at a given CO_2 concentration determine the net photosynthetic rate.

A major objective of this study was to determine whether photosynthetic adjustment occurs in field-grown trees after long-term exposure to elevated CO_2 . Based on our conceptual model (Luo et al. 1994, Griffin and Seemann 1996), which assumed that the initial response to elevated CO_2 was an increase in net photosynthesis and carbohydrate production, the occurrence of photosynthetic adjustment in ponderosa pine should be observed as: (1) reduced photosynthetic capacity, (2) decreased Rubisco activity and Rubisco content, (3) increased leaf mass per unit leaf area, and (4) decreased mass-based leaf nitrogen concentration.

Materials and methods

Growth conditions

This study was conducted at the USDA Forest Service Institute of Forest Genetics (Placerville, CA) on a level uniform site at an elevation of 843 m with a soil of Aiken clay loam, a Xeric Haplohumult derived from andesite. Before this study, the site was occupied by a sparse grass cover and the surrounding vegetation consisted primarily of mature ponderosa pine trees. Site preparation consisted of disking the soil to a depth of 25 cm. In 1991, hexagonal open-top chambers, each 3.6 m in diameter, were constructed of clear acrylic plastic sheeting to a height of 2.5 m with frustrums of the same material extending above the vertical chamber walls. Chamber CO₂ partial pressures were maintained at ambient CO₂ (35 Pa) and elevated CO₂ (70 Pa) for 24 h d⁻¹ for the entire six-year experimental period. Soil N conditions were maintained at ambient N (no additional N added) or high N (added 20 g N m⁻² yr⁻¹); N was added annually as (NH₄)₂SO₄ (Walker et al. 1997). Three open-top chambers were maintained at each CO₂ (ambient, elevated) and N (ambient, elevated) treatment.

Before planting, half-sib ponderosa pine seeds (Eldorado County, CA seed source) were stratified at 3 °C for 30 days and then sterilized in 10% H₂O₂ for 10 minutes. In May 1991, 66 pretreated seeds were sown directly in the soil, maintaining equal spacing between each planted seed, in each chamber (6.48 seeds m⁻²) and irrigation was applied as necessary to induce germination. By the end of the first growing season, seedlings had been thinned to 22 equally spaced seedlings per chamber (2.16 seedlings m⁻²).

²). Subsequent harvests and tree thinning procedures, systematically applied to maintain equal spacing between remaining trees, reduced the number of trees in each chamber to six ($0.59 \text{ trees m}^{-2}$) during our measurements in the sixth growing season. Tree seedlings were grown under approximately ambient conditions of temperature and light. Irrigation water was delivered to each chamber in one 30-minute period (approximately 0.7 cm water) on each of three days per week from April through October; during other periods, chambers were exposed to ambient precipitation conditions.

Gas exchange

Net photosynthetic rate vs. internal CO_2 partial pressure (A_{Ci}) response curves were measured on five fully expanded needles from current-year fascicles on 14 trees (2-3 trees per chamber) in each CO_2 treatment. A_{Ci} curves were conducted on randomly selected trees during a three day period in September 1996 using a LI-6400 portable photosynthesis system (LiCor Inc., Lincoln, NE). All gas exchange measurements were conducted on clear days in open-top chambers under saturating conditions of PPFD (at least $1200 \mu\text{mol m}^{-2} \text{ s}^{-1}$; Edwards 1989). A_{Ci} curves were measured on needles at twelve CO_2 partial pressures ranging from 0 to 120 Pa CO_2 (C_a was controlled), needle temperature was maintained at 28°C , and needle-to-air VPD was $2.87 \pm 0.06 \text{ kPa}$ ($n=28$), reflecting ambient water vapor conditions. Net photosynthetic rates were calculated on a total needle surface area basis estimated using geometric measurements (Lewis et al. 1994). Net assimilation at the growth CO_2 partial pressure (A_{growth}) and the maximum assimilation rate (A_{max}) for needles were determined from A_{Ci} curves. A_{growth} was

determined at growth C_a and A_{max} was the maximum A observed on each ACi curve under saturating light and CO_2 conditions.

Relative stomatal limitation (RSL) is an estimate of the reduction in net assimilation rate resulting from the reduction in C_i (internal CO_2 partial pressure) relative to C_a (atmospheric CO_2 partial pressure) due to the cumulative resistances to CO_2 diffusion between the atmosphere and the mesophyll. RSL was calculated from ACi curves for trees grown in atmospheric CO_2 partial pressures of 35 and 70 Pa using the following equation (Farquhar and Sharkey 1982):

$$\text{Relative stomatal limitation} = (1 - A/A_o) \times 100$$

where A is the net assimilation rate at the growth C_a (35 or 70 Pa), and A_o is the net photosynthetic rate at a C_a resulting in a C_i equal to the growth C_a . Under these conditions, A_o is the net photosynthetic rate that would occur if there was no resistance to diffusion of CO_2 . The ratio of internal to atmospheric CO_2 concentration (C_i/C_a) was calculated at the growth C_a .

Biochemical model of photosynthesis

The biochemistry of photosynthesis was modeled following Harley and Sharkey (1991) as modified by Lewis et al. (1994). The model was used to estimate two parameters potentially limiting to photosynthesis from the ACi curves: (1) V_{cmax} , the maximum carboxylation rate of Rubisco, and (2) J_{max} , RuBP regeneration capacity mediated by

maximum electron transport rate. Values for V_{cmax} , J_{max} and R_d (rate of nonphotorespiratory CO_2 efflux occurring in the light) were obtained as in Lewis et al. (1994). At saturating PPFD and C_i less than 20 Pa, RuBP concentrations were assumed to be non-limiting and that the rate of carboxylation was a function of V_{cmax} and R_d . Best fit values were calculated for V_{cmax} and R_d by using non-linear least-squares regression techniques on data collected at C_i less than 20 Pa. Using the values of V_{cmax} and R_d calculated for each ACi curve, it was possible to obtain best fit estimates for J_{max} at C_i where RuBP regeneration capacity was limiting, using non-linear least squares regression techniques to fit entire ACi curves using Data Desk 5.0 (Data Description Inc., Ithaca, NY). Glycerate re-entry into the chloroplast during photorespiration was assumed to be 75% (Harley and Sharkey 1991).

Rubisco extraction and assay

Current-year needles were collected for Rubisco extraction and analysis concurrent with needles collected for chlorophyll, nitrogen and TNC. Needles were immediately extracted in a buffer solution (Tissue et al. 1993), then frozen in liquid N and stored until analyzed. Photosynthetic photon flux density (PPFD) was at least $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$ and leaf temperature was 28°C during harvest. Initial and total (fully activated) Rubisco activity were measured at 25°C and analyzed spectrophotometrically by measuring the rate of oxidation of NADH in an enzyme-coupled assay (Tissue et al. 1993). Rubisco activation state was calculated from the ratio of initial activity to total activity. Rubisco content was determined by measuring ^{14}C -carboxyarabinitol biphosphate (^{14}C -CABP) binding to the

catalytic sites of Rubisco (Sharkey et al. 1986). Rubisco N was calculated from the amount of Rubisco protein assuming that 16.67% of Rubisco is N (Ridley et al. 1967, Steer et al. 1968). Activity of Rubisco on a molar basis was calculated by dividing Rubisco activity by Rubisco content in the needles.

Leaf properties

Nitrogen content was determined on needles dried at 70 °C, ground in a Wiley mill, digested using a microKjeldahl technique, and measured using a Technicon Traacs 800 autoanalyzer (Lowther 1980). Soluble sugar content and starch content of needles were determined colorimetrically using a phenol-sulfuric acid technique (Tissue & Wright 1995). Total nonstructural carbohydrate (TNC) was calculated as the sum of soluble sugar and starch. Total chlorophyll content was determined by grinding needles in liquid N, extracting needles twice with 80% (v/v) acetone, centrifuging for one minute and measuring the absorbance of the supernatant at 646.6 and 663.6 nm (Porra et al. 1989).

Statistical analyses

The effects of CO₂ treatment and N treatment on all measured parameters were examined by performing analysis of variance (ANOVA) on least squares estimates of treatment means using linear models (Data Description Inc., Ithaca, NY). In all cases, soil N treatment had no significant ($P < 0.05$) effect and there were no significant CO₂ x N interactions for measured parameters. Therefore, all N data were pooled and an ANOVA

on least squares estimates of treatment means using linear models was used to test the effect of CO₂ treatment on all parameters. Least-squares estimates of model parameters were calculated from individual ACi curves by the non-linear regression model fitting function in Data Desk 5.0 (Data Description Inc., Ithaca, NY). Treatment effects were considered significant if $P < 0.05$.

Results

Photosynthesis

Trees grown in elevated CO₂ had 53% higher net photosynthetic rates than ambient CO₂ grown trees when measured at their respective growth CO₂ partial pressures (Table 1). However, growth in elevated CO₂ significantly reduced photosynthetic capacity, as $A_{\max}$ declined 20% compared with ambient CO₂ trees (Table 1). Despite a reduction in photosynthetic capacity in elevated CO₂ grown trees, photosynthesis was CO₂-saturated at a C_i of approximately 60 Pa and the CO₂ compensation point was approximately 6 Pa for trees in both CO₂ treatments. There were apparent reductions in stomatal limitation to photosynthesis when ponderosa pine trees were grown in elevated CO₂. Values of relative stomatal limitation (RSL) indicated that resistance to diffusion of CO₂ through stomata reduced photosynthesis by 39% for trees grown at ambient CO₂, but this stomatal limitation to photosynthesis was reduced to 28% when trees were grown at elevated CO₂ (Table 1). The ratio of C_i/C_a , which reflects changes in the relationship between stomatal

conductance and the biochemical capacity for photosynthesis (Ball and Berry 1982), was not affected by growth CO_2 partial pressure (Table 1).

Biochemical model of photosynthesis

Analyses of ACi curves using a biochemical model of photosynthesis indicated a 36% reduction in maximum Rubisco carboxylation efficiency (V_{cmax}) and a 21% reduction in RuBP regeneration capacity (J_{max}) for trees grown in elevated CO_2 (Table 1). The $J_{\text{max}}:V_{\text{cmax}}$ ratio was significantly increased 24% ($P=0.0002$), from 1.99 in ambient CO_2 to 2.46 in elevated CO_2 , and is consistent with observations for a wide range of species (Wullschleger 1993). Therefore, N was apparently reallocated away from Rubisco toward proteins involved in electron transport. The rate of nonphotorespiratory CO_2 efflux occurring in the light (R_d) was not affected by CO_2 treatment and for trees in both CO_2 treatments was approximately 1% of A_{growth} (Table 1).

Rubisco activity and content

Biochemical measurements of Rubisco activity were consistent with values generated by the biochemical model of photosynthesis. Rubisco activity was reduced 38% in elevated CO_2 grown trees (Table 2), comparable with a 36% reduction in V_{cmax} (Table 1). Rubisco activation state was approximately 72% for all trees and did not differ between CO_2 treatments (Table 2). Rubisco molar activity also did not differ between CO_2 treatments and was approximately $23 \text{ mol CO}_2 \text{ mol Rubisco}^{-1} \text{ s}^{-1}$ (Table 2). Rubisco content was

reduced 38% in elevated CO₂ grown trees, declining in concert with Rubisco activity (Table 2). Differences in Rubisco content expressed on a nitrogen and chlorophyll basis indicate changes in resource allocation due to growth in elevated CO₂ (Table 2). Significantly less N was allocated to Rubisco protein in needles of trees grown in elevated CO₂ (2.4% of total N) compared with trees in ambient CO₂ (3.5% of total N). Similarly, Rubisco content expressed on a chlorophyll basis, used to estimate shifts in allocation between Rubisco and thylakoid components (Evans and Terashima 1987), was reduced 19% by growth in elevated CO₂ (Table 2). Therefore, relatively less N was allocated to Rubisco protein and relatively more N to light harvesting components in needles of trees grown in elevated CO₂, in agreement with $J_{\max}:V_{c\max}$ ratios generated from ACi curves.

Leaf Properties

Total chlorophyll content declined 24% in needles of trees grown in elevated CO₂ (Table 3), which was similar to the 21% reduction in $J_{\max}$ due to elevated CO₂ (Table 1). This suggests that the reduction of RuBP regeneration capacity mediated by electron transport rate ($J_{\max}$) observed at elevated CO₂ may largely be due to reductions in chlorophyll content. Leaf mass per unit area was not significantly affected by growth in elevated CO₂ (Table 3). Elevated CO₂ reduced needle N on a mass basis by 14% (Table 3). When calculated on a TNC-free basis (data not shown) to remove possible carbohydrate dilution effects, leaf N on a mass basis was still significantly reduced ($P=0.0002$). However, there was not a significant effect of elevated CO₂ on needle N expressed on an area basis,

which is the product of mass-based N content and leaf mass per unit area. Similarly, growth in elevated CO₂ did not significantly affect needle sugar, starch or TNC content (Table 3).

Discussion

Net photosynthesis of ponderosa pine needles was stimulated 53% at the end of the sixth year of exposure to elevated atmospheric CO₂. In a comprehensive literature review of tree response to growth in elevated CO₂ in open top chambers in the field, Norby et al. (1998) found a mean photosynthetic enhancement of 67% in elevated CO₂, with little difference in response between deciduous and coniferous trees. In most trees, net photosynthesis was stimulated 40-80% by growth in elevated CO₂, with the lowest stimulation for non-shade grown trees being 20% in *Mangifera indica* (Goodfellow et al. 1997). In loblolly pine (*Pinus taeda*), net photosynthesis of trees grown in the field in conditions that did not limit rooting volume was also stimulated by growth in elevated CO₂ (Ellsworth et al. 1995, Liu and Teskey 1995, Teskey 1995, Lewis et al. 1996), even after four years of CO₂ exposure (Tissue et al. 1997). In general, net photosynthesis is enhanced in trees grown in elevated CO₂ and that enhancement is maintained for long periods.

Despite increased net photosynthesis at the growth CO₂ partial pressure, there was a reduction in the photosynthetic capacity of ponderosa pine trees grown in elevated CO₂. Reductions in photosynthetic capacity of elevated CO₂ trees were evident as analyses of ACi curves indicated significant reductions in maximum photosynthetic rate (A_{max} , 20%),

Rubisco carboxylation capacity (V_{cmax} , 36%), and electron transport capacity (J_{max} , 21%). In addition, the reduction in photosynthetic capacity due to growth in elevated CO_2 was accompanied by reductions in various photosynthetic components, including total chlorophyll, Rubisco protein content, and mass-based leaf nitrogen concentration. These results are similar to the 9-23% reduction in photosynthetic capacity observed during the fourth growing season in field-grown *Betula pendula* exposed to elevated CO_2 (Rey and Jarvis 1998) and in middle canopy leaves of *Populus tremuloides* (Kubiske et al. 1997). However, these results contrast with other studies on field-grown trees that indicate no change in photosynthetic capacity with long-term exposure to elevated CO_2 (Gunderson et al. 1993, Teskey 1995, Kellomaki and Wang 1996, Scarascia-Mugnozza et al. 1996, Goodfellow et al. 1997). Although reduction in photosynthetic capacity is most common in pot-grown trees (cf. Gunderson and Wullschlegel 1994, Curtis and Wang 1998), our results and those of Rey and Jarvis (1998) clearly indicate that photosynthetic adjustment can occur in trees growing in the field with no apparent restriction in root growth.

Seasonal effects have been shown to influence the photosynthetic response to elevated CO_2 (El Kohen and Mousseau 1994, Curtis et al., 1995 Lewis et al. 1996, Tissue et al. 1997, Rey and Jarvis 1998). In two deciduous trees, *Castanea sativa* (El Kohen and Mousseau 1994) and *Betula pendula* (Rey and Jarvis 1998), photosynthetic adjustment to elevated CO_2 increased as the growing season progressed. In loblolly pine, the relative enhancement of photosynthesis due to elevated CO_2 was greater in the summer (60-130% increase) and lower in the winter (14-44% increase), reflecting a strong seasonal effect of temperature on photosynthesis (Lewis et al. 1996, Tissue et al. 1997). In addition, reduced sink strength during periods of minimal growth may also reduce the

photosynthetic response of trees to elevated CO₂. Therefore, the large reduction in photosynthetic capacity and associated photosynthetic components observed in ponderosa pine in late summer may be greater than the photosynthetic adjustment expected earlier in the growing season. Nonetheless, photosynthetic adjustment was observed in *Betula pendula*, an indeterminate growth species, in mid-season (Rey and Jarvis 1998), further suggesting that at least some degree of photosynthetic adjustment in ponderosa pine probably occurred earlier in the growing season.

Reductions in sink activity have been proposed as explanations for the reduction in photosynthetic capacity observed in plants grown in elevated CO₂ (Arp 1991, Stitt 1991, Thomas and Strain 1991). Sink limitation occurs when carbohydrate production exceeds carbohydrate utilization, which results in sugar accumulation that can trigger a feedback inhibition of the transcription of photosynthetic proteins (Sheen 1990, Cheng et al. 1998). If photosynthetic protein production is reduced, then triose phosphate production and utilization are also reduced (Herold 1980, Paul and Driscoll 1997). Sink limitation is often suggested as a mechanism that reduces Rubisco activity and Rubisco content during long-term exposure to elevated CO₂ (Stitt 1991, Luo et al. 1994). In this study, reductions in Rubisco activity, Rubisco content and RuBP regeneration capacity in elevated CO₂ were independent of leaf carbohydrate concentration, which was not affected by growth in elevated CO₂. Photosynthate production in ponderosa pine apparently did not exceed carbohydrate utilization. Therefore, sink limitation, operating through total soluble sugar or starch concentration, does not account for the long-term reduction in biochemical factors in ponderosa pine trees exposed to elevated CO₂, as has

also been observed for loblolly pine (Lewis et al. 1996) and silver birch (Rey and Jarvis 1998).

Photosynthetic adjustment to elevated CO_2 may also be regulated by nitrogen limitation induced by increased growth and subsequent increased nitrogen demand (cf. Sage and Reid 1994). Since up to 80% of total leaf nitrogen is associated with photosynthesis, including proteins, chlorophyll and membrane constituents, a reduction in leaf N concentration may adversely affect photosynthesis (Sage and Reid 1994). In ponderosa pine, there was a reduction in mass-based leaf N of 14%, similar to the reduction of 19% found for a wide range of woody plants (Cotrufo et al. 1998). In apparent response to reduced leaf N, there was reallocation of N within the photosynthetic apparatus to balance generally non-limiting processes at elevated CO_2 (Rubisco carboxylation) with generally limiting processes (RuBP regeneration mediated by electron transport capacity). Although there were reductions in the absolute amounts of Rubisco protein and total leaf chlorophyll, relatively more N was allocated to light harvesting components and proteins involved in electron transport and relatively less N was allocated to Rubisco protein in trees grown in elevated CO_2 . Similar results have been observed in loblolly pine (Tissue et al. 1993, 1996), but not in other plants (Woodrow 1994, Medlyn 1996, Rey and Jarvis 1998). In transgenic tobacco, Quick et al. (1991) reduced Rubisco activity by 49% with only a 14% reduction in photosynthesis when grown in elevated CO_2 . These results suggest that in the presence of elevated CO_2 , many plants have excessive Rubisco capacity, perhaps because Rubisco also functions as a form of N storage (Millard 1988), and therefore reductions in N investment in Rubisco may occur with little effect on net photosynthesis.

The effect of long-term growth in elevated CO₂ on morphological characteristics is generally not well studied. In some studies however, growth in elevated CO₂ has been shown to increase the number of layers of leaf mesophyll (Vu et al. 1989), increase leaf thickness (Leadley et al. 1987, Radoglou and Jarvis 1990), and increase cell and chloroplast volume (Robertson and Leech 1995). Combined, increases in these morphological factors increase leaf mass per unit area, which may be used as a measure of morphological adjustment to growth in elevated CO₂ (Luo et al. 1998). In ponderosa pine, there was no change in leaf mass per unit area due to growth in elevated CO₂. When leaf mass per unit area was calculated on a TNC-free basis, to remove possible confounding effects of carbohydrate content, there still was no effect of elevated CO₂, suggesting that morphological adjustment did not occur. These results are in contrast with increased leaf mass per unit area observed in a variety of herbaceous plants (cf. Luo et al. 1998), field-grown loblolly pine (Tissue et al. 1996), *Quercus robur* and *Prunus avium* (Atkinson et al. 1997) exposed to elevated CO₂, but in agreement with other studies showing no response (cf. Luo et al. 1998). In six-month old greenhouse-grown ponderosa pine seedlings exposed to elevated CO₂, the amount of mesophyll tissue was reduced (Pushnik et al. 1995) indicating that ponderosa pine has the capacity for morphological adjustment, but our study shows that it is not always exhibited.

In summary, net photosynthesis was enhanced in ponderosa pine trees grown in elevated CO₂ at the end of the sixth growing season, despite reductions in photosynthetic capacity. The positive net photosynthetic response of ponderosa pine trees to elevated CO₂ reflected greater relative increases in Rubisco sensitivity than decreases due to biochemical adjustments, as predicted by the conceptual model (Luo et al. 1994). That is,

elevated CO₂ increased the rate of Rubisco carboxylation at growth CO₂ concentration to a greater degree than it decreased biochemical capacity, observed as reductions in V_{cmax} , J_{max} , Rubisco activity and content, N and chlorophyll, thereby increasing net photosynthesis. Net photosynthesis was unaffected by morphological adjustments since there was no change in leaf mass per unit area in elevated CO₂. An apparent positive response of photosynthetic adjustment in elevated CO₂ was the redistribution of N within the photosynthetic system to balance limiting and non-limiting processes. These results clearly demonstrate that trees, without apparent limitations to root growth, may exhibit photosynthetic adjustment responses in the field after long-term exposure to elevated CO₂. Information from long-term studies on photosynthetic adjustment responses in trees will improve our ability to predict annual global carbon influx into terrestrial ecosystems and improve models of forest ecosystem response to global climate change.

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Table 1. Gas exchange parameters calculated from ACi response curves measured for needles on trees grown at ambient (35 Pa) and elevated (70 Pa) CO₂. ACi curves were measured in September in the field under saturating conditions of PPFD (at least 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$), 28 °C needle temperature and ambient vapor pressure deficit. Values are means $\pm$ SEM for individual trees in six chambers in each CO₂ treatment. The significance of the CO₂ treatment effect is indicated by the P-value or as non-significant (ns). Relative change is the percentage increase or decrease due to growth in elevated CO₂.

Parameter	CO ₂ Partial Pressure		P-value	Relative Change (%)
	Ambient (35 Pa)	Elevated (70 Pa)		
A_{growth} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	5.76 (0.31)	8.83 (0.50)	0.0001	53
A_{max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	13.88 (0.87)	11.10 (0.68)	0.0180	-20
R_d ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	0.047 (0.047)	0.102 (0.027)	ns	ns
$V_{c_{\text{max}}}$ ($\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$)	22.7 (2.1)	14.5 (0.9)	0.0011	-36
J_{max} ($\mu\text{mol E m}^{-2} \text{s}^{-1}$)	45.2 (2.5)	35.7 (2.2)	0.0086	-21
RSL (%)	39.0 (1.6)	27.8 (1.7)	0.0001	-29
C_i/C_a	0.60 (0.01)	0.61 (0.01)	ns	ns

Table 2. Properties of Rubisco for needles on trees grown at ambient (35 Pa) and elevated (70 Pa) CO₂. Values are means $\pm$ SEM for individual trees in six chambers in each CO₂ treatment. The significance of the CO₂ treatment effect is indicated by the P-value or as non-significant (ns). Relative change is the percentage increase or decrease due to growth in elevated CO₂.

Parameter	CO ₂ Partial Pressure		P-value	Relative Change (%)
	Ambient (35 Pa)	Elevated (70 Pa)		
Rubisco activity ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	15.3 (1.5)	9.5 (0.6)	0.0017	-38
Rubisco molar activity ($\text{mol CO}_2 \text{ mol rubisco}^{-1} \text{s}^{-1}$)	23.2 (2.3)	23.2 (1.4)	ns	ns
Rubisco activation state (%)	72.1 (1.7)	72.9 (1.7)	ns	ns
Rubisco content (mg m^{-2})	362.7 (34.7)	225.1 (15.1)	0.0012	-38
(mg mg N^{-1})	0.35 (0.03)	0.24 (0.01)	0.0016	-31
(g mmol chl^{-1})	1.32 (0.10)	1.07 (0.08)	0.0536	-19

Table 3. Characteristics of needles on trees grown at ambient (35 Pa) and elevated (70 Pa) CO₂. Values are means $\pm$ SEM for individual trees in six chambers in each CO₂ treatment. The significance of the CO₂ treatment effect is indicated by the P-value or as non-significant (ns). Relative change is the percentage increase or decrease due to growth in elevated CO₂.

Parameter	CO ₂ Partial Pressure		P-value	Relative Change (%)
	Ambient (35 Pa)	Elevated (70 Pa)		
Total chlorophyll ($\mu\text{mol m}^{-2}$)	286.5 (28.4)	217.3 (14.7)	0.0397	-24
Leaf mass/area (g m^{-2})	91.0 (2.8)	97.7 (4.0)	ns	ns
Nitrogen (mg g^{-1})	11.34 (0.41)	9.71 (0.29)	0.0033	-14
Nitrogen (g m^{-2})	1.03 (0.04)	0.95 (0.05)	ns	ns
Sugar (%)	4.73 (0.22)	4.78 (0.19)	ns	ns
Starch (%)	3.45 (0.20)	3.62 (0.17)	ns	ns
TNC (%)	8.11 (0.24)	8.40 (0.23)	ns	ns